



1 Soil-biodegradable plastic films do not decompose in a lake sediment over 9 months  
2 of incubation

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15 Keywords: plastic, soil, biodegradable, mulch foil, agriculture, lake sediment

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17

## 18 **Abstract**

19 Agriculture relies heavily on the use of plastic mulch films, which increase crop yields  
20 and can lower water demands. In recent years, soil-biodegradable mulch films have  
21 been marketed to replace the non-biodegradable, conventional polyethylene-based  
22 mulch films. These biodegradable mulch films are designed to be ploughed into the  
23 soil after use to biodegrade *in situ* by soil microorganisms. However, research has  
24 shown that part of the mulch film material may be transported from the fields to  
25 neighboring environments, including aquatic ecosystems. Research on potential  
26 biodegradation of soil-biodegradable plastics in freshwater habitats is lacking. Here,  
27 we investigated the mineralization of soil-biodegradable agricultural mulch films in  
28 freshwater lake sediments of Lake Lucerne, Switzerland. Two types of commercial  
29 soil-biodegradable mulch films were incubated within lake sediment cores, along with  
30 traditional PE plastic, and the production of CO<sub>2</sub> and CH<sub>4</sub> was followed over time  
31 relative to non-plastic containing control sediments. After the 40-week incubation  
32 period, films were visually intact and showed no signs of mineralization. Gas  
33 analyses showed no additional production of either CO<sub>2</sub> or CH<sub>4</sub> in the degradable  
34 mulch film incubations, compared to control or PE plastic incubations. We conclude  
35 that these two used soil biodegradable mulch films have a low biodegradability in  
36 lake sediments, likely reflecting that the microbial community structure in the lake  
37 sediment lacks active microbial degraders. Our results highlight the importance of  
38 preventing transport of soil-biodegradable mulch films from agricultural soils to  
39 surrounding aquatic environments.

40

41



## 42 Introduction

43

44 Part of the solution to overcoming plastic pollution in the environment is to use  
45 biodegradable plastic instead of conventional plastics in applications in which  
46 plastics are used in the open environment and for which complete recollection of the  
47 plastic after use is impossible. A picture-case example are thin agricultural mulch  
48 films. Modern agriculture relies heavily on the use of plastic mulch films to elevate  
49 soil temperature and maintain soil moisture, to provide protection and to prevent  
50 weed growth. Conventional mulch films are composed of polyethylene (PE). If these  
51 films are thin, they cannot readily be reused nor recycled after application, which  
52 means they need to be incinerated or landfilled after single use (Serrano-Ruiz et al.,  
53 2021). Such a short life-cycle of plastic products is undesirable, and both incineration  
54 and landfilling are considered undesirable end of life treatment options. Another  
55 disadvantage of conventional thin (<25 µm) mulch films is that they cannot easily be  
56 re-collected entirely after their application, leaving PE plastic residues in the soil.  
57 Because PE films persist, these residuals building up in soils over time, eventually  
58 harming agricultural productivity (Gao et al., 2019). Furthermore, the films may

\*59 slowly decompose into microplastics. Both ~~both~~ macro- and microplastics have been  
60 shown to be transported by wind and water, away from the initial application site,  
61 towards waterways, lakes and coastal seas (Egessa et al., 2020; Ren et al., 2021;  
62 Yang et al., 2022), where they have been shown to cause harm to the ecosystem  
63 (Galloway et al., 2017; Hale et al., 2020).

64 In an attempt to limit soil plastic pollution, the agricultural industry has developed  
65 soil-biodegradable mulch films as substitutes of conventional films. These soil-  
\*66 **biodegradable films are composed of polymers** designed to be degradable by soil  
67 microorganisms under the moisture and temperature conditions found in the soil  
68 environment. A standard has been set by the EU (2018 EU standard EN 17033) for  
69 the biodegradability of mulch films named as such. In general, the biodegradation of  
70 the soil-biodegradable mulch films (hereafter referred to as BD-films) involves two  
71 stages: i. Breakdown of the large pieces into small organic molecules by abiotic or  
72 biological (enzymatic) processes. II. Uptake and metabolic use of these organic  
73 molecules by microorganisms under conversion into CO<sub>2</sub> and microbial biomass  
74 (Zumstein et al., 2018).

75 Soil-biodegradable mulch films are designed to be biodegraded in soil. It is, however,  
76 possible that fragments of the of BD-films may be transported away from agricultural  
77 soils before being completely biodegraded. While we are not aware of studies  
78 specifically on the transport of BD- films, transport of conventional mulch films by  
79 wind or water has been observed (Dris et al., 2015; Ren et al., 2021; Yang et al.,  
80 2022).

81 Earlier studies have shown successful biodegradation of BD-films in soil  
82 environments (Li et al., 2014; Sintim et al., 2020), with complete biodegradation  
83 being predicted to take several years. Research on the aquatic biodegradation of  
84 BD-plastics is predominantly focused on the marine realm. There, several studies  
85 have shown microbial colonization on BD-plastics (Eich et al., 2015; Nauendorf et  
86 al., 2016; Morohoshi et al., 2018), and some showed a disintegration of the plastics  
87 over time (Briassoulis et al., 2020; Lott et al., 2020; Wei et al., 2021), although these  
88 studies were not performed on mulch films, but on other BD-plastic products, such  
89 as bags. The transferability of studies on different BD-plastics is limited, but as there  
90 are currently no studies on BD mulch films in the aquatic environment available, we  
91 still introduce these studies on other BD plastic products here. All the above

I suggest to add here other compounds that have been used to make BD-films, like oil- and wax-coated paper. I also suggest to add respective citations.



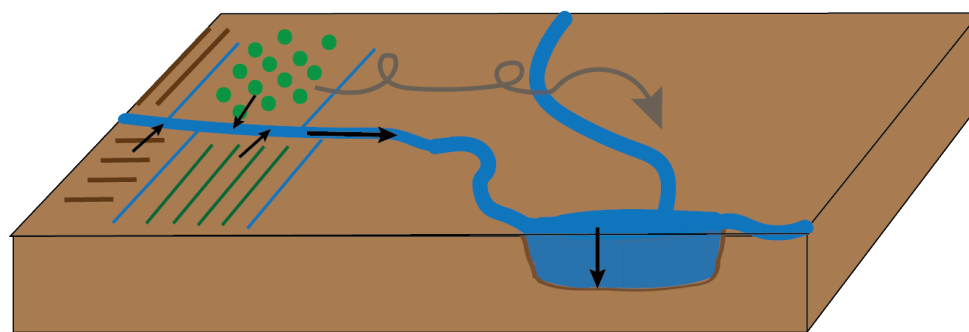
92 presented studies are disintegration studies, which generally cannot distinguish  
93 whether BD-plastics merely physically degraded into smaller particles (i.e.,  
94 fragmentation), or whether they have been microbially biodegraded, resulting in  
95 microbial biomass and CO<sub>2</sub> and/or CH<sub>4</sub> production, which makes an assessment of  
96 the mineralization of the BD plastics difficult. A recent study by Lott et al. 2021 (Lott  
97 et al., 2021) measured marine biodegradation of BD plastic components (PHB,  
98 PBSe) by CO<sub>2</sub> measurements, in experiments with marine sediments. There, they  
99 found a 116 and 222 days in incubation experiments under benthic and eulittoral  
100 marine conditions.

101

102 Here, we determine the biodegradability of BD- films in freshwater lake sediments by  
103 performing sediment incubation studies with two types of soil-biodegradable, and  
104 one traditional PE plastic mulch films. Our results show that none of the tested films  
105 biodegraded over the 40 weeks incubation period: we detected no excess production  
106 of CO<sub>2</sub> or methane in the incubation experiments with BD-plastics as compared to  
107 PE-films and plastic-free control sediments. Furthermore, there were no signs of any  
108 physical degradation of any of the films after the 40 weeks incubation. Our findings  
109 suggest that the conditions in the tested sediment, likely including abiotic factors  
110 (unfavorable temperature and water chemistry) and biotic factors (low abundance  
111 and activity of BD-MF degrading microorganisms) impaired biodegradation in the  
112 lake. Our findings suggest significant higher stability of soil-biodegradable mulch  
113 films in lake sediments than soils, highlighting the need for measures to control off-  
114 field transport of soil BD-films.

115

116



117

118 Fig. 1. Transport of plastics from agricultural sources to lake sediments, either via  
119 water ways or via wind transport.

120



121

## 122 **Methods**

123

### 124 Sediment collection

125 Sediment cores were taken from Lake Lucerne (Switzerland) at 46°59'38"N  
126 8°20'57"E in June 2021. Multiple deployments with a multicorer device were used to  
127 collect the required number of sediment cores, which were later randomly distributed  
128 over the treatments. Sediments were collected into 10 cm diameter, transparent  
129 butyrate plastic core liners. All sediment cores were brought into a climate room of  
130 10°C (to mimic the temperature of Lake Lucerne bottom waters, see (Fiskal et al.,  
131 2019)) within 2 hours after core collection.

132

### 133 Gas analysis incubation experiment

134 To determine the production of CO<sub>2</sub> and CH<sub>4</sub> from the sediments, 16 cores were  
135 setup for incubation studies in the 10°C climate room. An overview of the  
136 experimental setup is presented in Fig. 2. Four cores did not receive any treatment  
137 (film-free control group), four cores were supplied with PE films, and eight with  
138 biodegradable mulch films purchased from two Swiss agricultural vendors: four cores  
139 received BD mulch films purchased from Sansonnens FG Frères SA (Rueyres-les-  
\* 140 Prés, Switzerland), called MF-S from here on, and four with biodegradable plastics  
141 purchased from Gvz-rossat AG (Otelfingen, Switzerland), called MF-R from here on.  
142 These plastics are classified as 'OK biodegradable soil' by TÜV Austria, and are  
143 composed mainly of PBAT and polylactic acid (PLA)(Nelson et al., 2019). The  
144 plastics (all kinds) were cut and weighted. Each core received 300 milligrams of  
145 plastics, folded multiple times to form a rectangular stack. To increase the surface  
146 area for degradation and to make the stacks of plastic film loose, rather than  
147 squeezed together, the plastics were cut into comb shaped pieces (see Fig. S1 for  
148 plastics weights and shape), which allowed sediment between the folded layers. Part  
149 of the water was removed from each core, to allow for an air headspace above the  
150 overlying water. The oxygen concentration in the headspace was not tracked, but  
151 results from earlier experiments with the same sediments showed oxygen was not  
\* 152 completely consumed over one year's time (van Grinsven et al., in prep. Data can be  
\* 153 made available to reviewers).

154 For a part of the cores, the stopper at the bottom was pushed upwards slightly (5-15  
155 cm) to decrease the water and headspace volumes above the sediment surface, to  
156 create more equal headspace volumes in each core. This type of cores was  
157 distributed over the treatments (2 per treatment). The procedure was not repeated  
158 for all used cores, because not all stoppers turned out to be tight enough to be  
159 pushed up and remain in position. All cores, pushed up or not, had a 7 cm height gas  
160 headspace (±550 cm<sup>3</sup>). Water headspace volumes varied between cores. One of the  
161 PE cores leaked water over the course of the experiment and was therefore  
162 discarded from the results. Films (biodegradable/PE) were pushed into the sediment  
163 surface (1-2 cm). Each of the cores was closed off with an adapted stopper, that  
164 contained two sampling ports, as specified in van Grinsven et al. (in prep). These  
165 stoppers allowed for non-invasive gas sampling over the course of the experiment.

\* 166 The cores were incubated in the dark at 10°C for 40 weeks. Gas samples (10 ml)mL  
167 were taken monthly. The removed gas was resupplied with N<sub>2</sub> gas to prevent  
168 underpressure in the cores. The volume of gas exchanged on a monthly basis was  
169 less than 2% of the gas headspace. Gas samples were pushed into 60 mL serum  
170 bottles containing N<sub>2</sub> gas, which were analyzed for CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O

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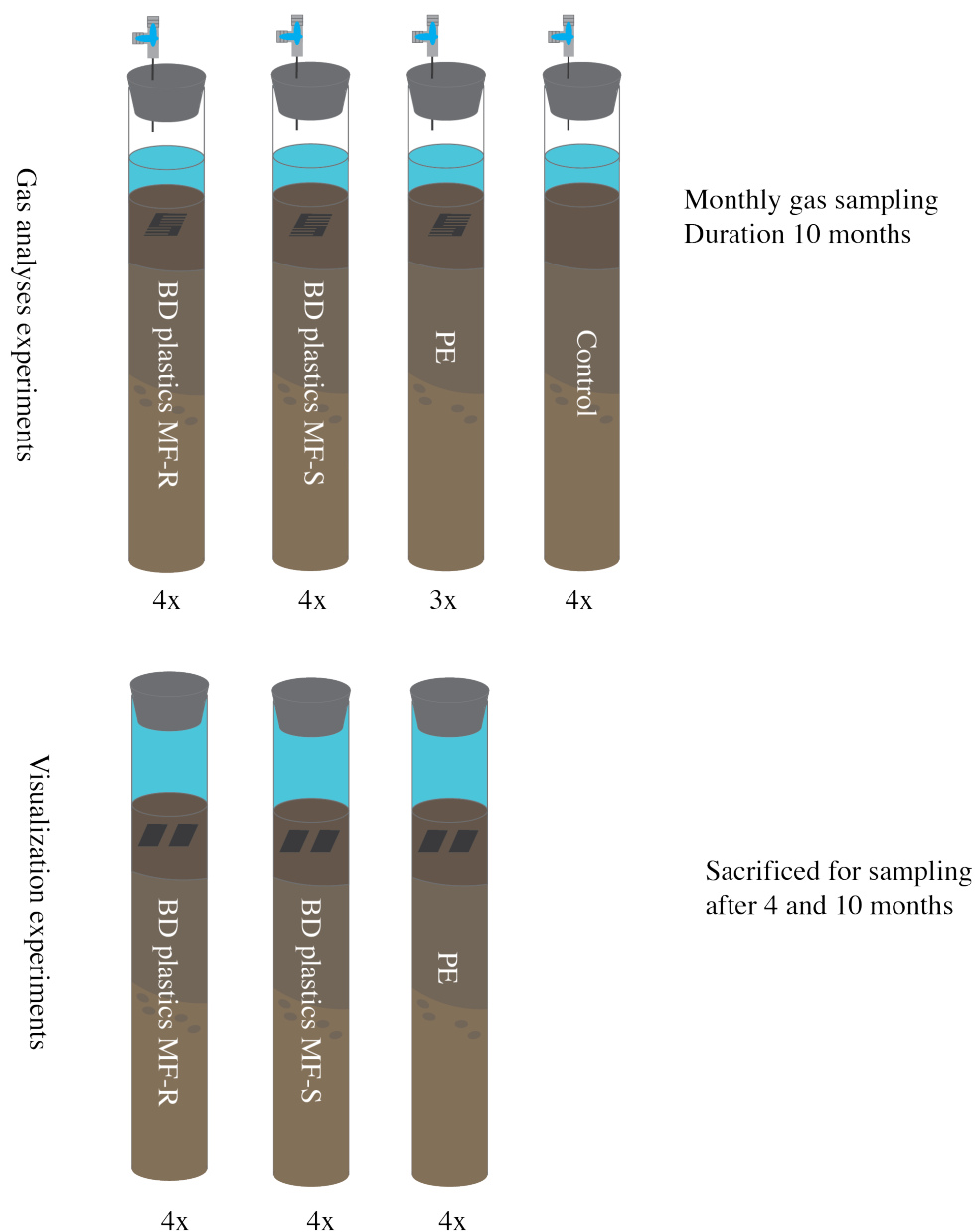


171 concentrations. Gas samples were analyzed by gas chromatography (GC; Agilent  
172 6890N, Agilent Technologies) using a Carboxen 1010 column (30 m x 0.53 mm,  
173 Supelco), a flame ionization detector and an auto-sampler (Valco Instruments Co.  
174 Inc.)  
175 The change in headspace CO<sub>2</sub> and CH<sub>4</sub> concentration over time was taken as a  
176 measure of biodegradation. The headspace CO<sub>2</sub> and CH<sub>4</sub> concentrations of all  
\* 177 cores of each treatment were averaged, ~~as shown in Fig. 3.~~ The data of each  
178 individual core is shown in the supplemental material (Fig. S2; S3).  
179  
180 Visualization incubation experiment  
181 Identical cores were used for the gas analysis and visualization experiments. 12  
182 cores were used for the visualization experiment: four cores received PE plastics,  
183 four cores MF-S BD-plastics, and four cores MF-R BD-plastics, as shown in Fig. 2.  
184 No control cores were included, as these experiments were purely used for  
185 visualization of the extracted plastics, and control experiments would therefore not  
186 result in any results, as nothing could have been visualized. As a smaller quantity of  
187 plastics could be added to the visualization experiments, the plastics did not need to  
188 be folded, and therefore, they were not cut either (Fig. S1). Two unfolded pieces of  
189 plastics were added to each core. No cores were pushed up and no headspace was  
190 created, the cores were filled with water up to the stopper, to cause minimal  
191 disturbance and to keep conditions close to *in situ* conditions. Bottom water  
\* 192 contained around 400 µM of oxygen at the start of the experiment. Earlier  
\* 193 experiments (van Grinsven et al., in prep, data available to reviewers on request)  
\* 194 showed limited oxygen consumption over the course of one year. Non-adapted  
195 stoppers were used, as these cores were not subsampled over the course of the  
196 experiment. Incubation occurred in parallel to the gas analysis experiment, at 10°C in  
197 the dark.  
198 After 18 weeks, two cores per treatment were sampled and sacrificed. The plastics  
199 were removed from the sediments with tweezers, pulled through the overlying water,  
200 and put into a 4% paraformaldehyde solution overnight at 4°C to fixate the samples.  
201 The samples were then rinsed twice with PBS 1x to remove paraformaldehyde, and  
202 then stored at -20°C until further usage. This was repeated after 40 weeks for the  
203 remaining sediment cores.  
204  
\* 205 (Biodegradable) plastic samples were used for microscopy after all samples were  
206 retrieved at the end of the experiments. Samples were stained with DAPI (1 mg/ml) mg L<sup>-1</sup>  
207 and placed on microscope slides with a mounting and anti-fading medium. The  
208 samples were viewed under a Leica fluorescence microscope with 100x and 400x  
209 magnification, to look for biofilm growth and visible degradation of the plastics.  
210  
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**Fig. 2.** Experimental setup. The gas analysis experiments contained a headspace to allow for gas sampling, via a sampling port. The visualization experiments had no gas headspace, the entire space above the sediment was filled with *in situ* bottom water. No control experiment was included in the visualization experiments, as these experiments were solely used for microscopy of the retrieved plastics (see methods). All experiments were performed at *in situ* (Lake Lucerne) temperature, in the dark. BD – Biodegradable; PE – polyethylene; MF-R and MF-S – Biodegradable mulch films of two Swiss companies (see methods).



## 221 Results

222

223 Concentrations of CO<sub>2</sub> and methane in the gas headspace of the sediment cores  
224 were followed over the course of the experiment, to assess plastic biodegradation.  
225 Given the organic composition of each of the plastics types, being composed of  
226 organic polymers, CO<sub>2</sub> and possibly CH<sub>4</sub> were expected to be key biodegradation  
227 products, alongside with microbial biomass. In aquatic environments, research on  
228 BD plastics has predominantly been focused on assessing degradation by visual  
229 assessments (photography, microscopy) or by repeated measurements of remaining  
230 plastic mass over time (Briassoulis et al., 2020; Lott et al., 2020). A downside of  
231 these approaches is that fragmented plastics, which have only been partially  
232 degraded and then form micro- and nanoparticles (shown to form out of both PE and  
233 BD plastics (Wei et al., 2021)), can easily be missed, and their mass is incorrectly  
234 counted as being completely degraded.

235 Overall, the change in CO<sub>2</sub> concentration over time did not differ significantly  
236 between any of the treatments, including the control (pairwise ANOVA with post-hoc  
237 analyses with a Bonferroni adjustment revealed that all the pairwise differences

\* 238 between groups were not statistically significantly different,  $p > 0.05$ ). **During the first**

\* 239 **20 weeks, the four treatments behave similarly**, with a steady increase in headspace

240 CO<sub>2</sub> concentration (Fig. 3). From 20 weeks to the termination at 40 weeks, the  
241 concentration change differed slightly between treatments, but variation within  
242 treatments was large, and the difference between groups not statistically significant,  
243 as described above. In the incubations with PE-films, the CO<sub>2</sub> concentration  
244 increased until week 30 and then stabilized. In the control experiment, this

\* 245 stabilization was observed from week 20 until week 40. **In both the BD-plastics**

\* 246 **treatments, a decrease in the CO<sub>2</sub> concentration relative to the preceding weeks was**

\* 247 **observed from week 20 to week 30**, after which the concentrations stabilized.

248 Individual cores, however, showed variations within the treatments, and the  
249 difference between treatments were not statistically significant, as stated above.

250 Overall, the results show that the BD-plastics treatments did not produce larger  
251 amounts of CO<sub>2</sub> than the control (no addition) treatment.

252 Methane production from all sediments, including the control treatment, remained  
253 around zero from week 0 to week 15. After week 15, the methane concentration in  
254 the PE treatments increased slightly (Fig. 3; more detailed view available in the  
255 supplemental information, Fig. S4). In the control experiment, methane

256 concentrations dipped slightly below the starting concentrations, but then increased  
257 from week 28 onwards, albeit with large variations between individual sediment

\* 258 cores. **Both BD-plastics treatments showed large increases in relative amounts of**

\* 259 **methane from week 20 onwards**, although the large variations between individual

\* 260 cores within the treatments lead to large, overlapping error bars. **The stabilization of**

\* 261 **the CO<sub>2</sub> concentration and the increase in CH<sub>4</sub> concentration in the BD-plastics**

\* 262 **experiments matched timewise, both occurring from week 20 onwards.**

263

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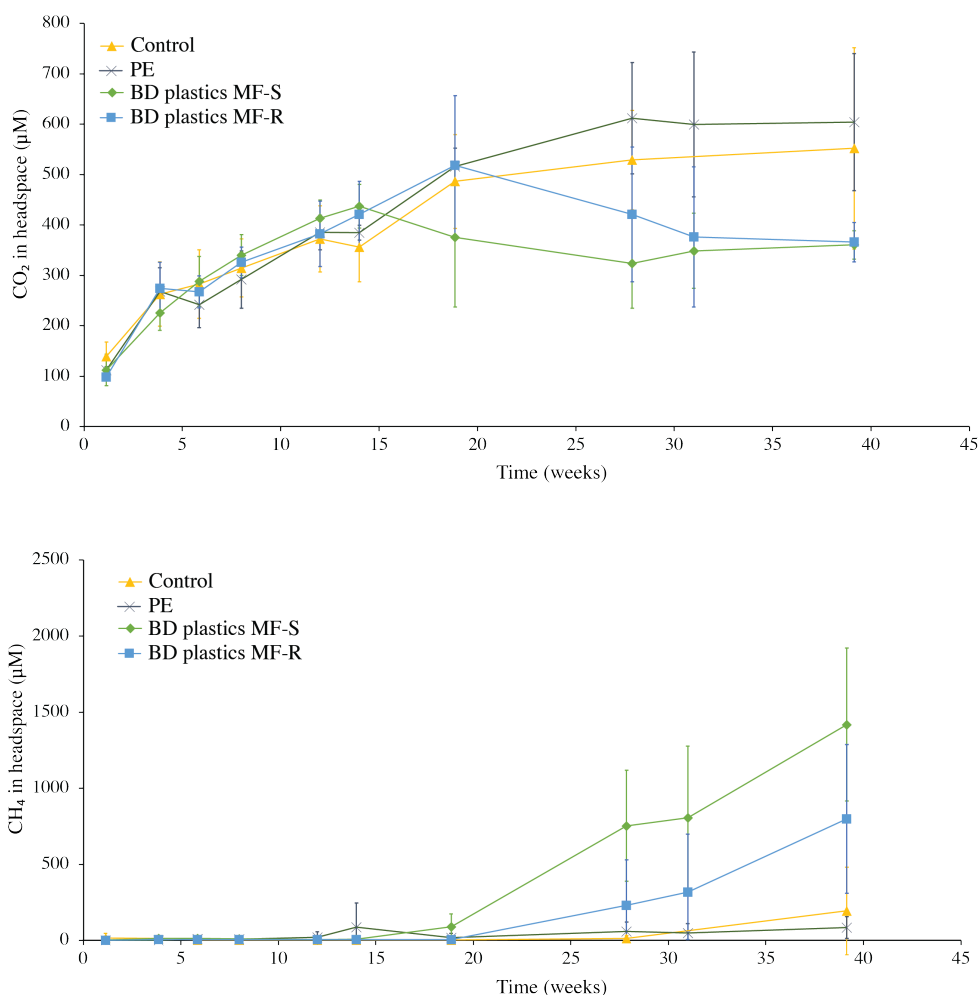
Review this comment  
because BD plastics MF-S  
sounds different from others  
after week 15.

I disagree in part, because  
CO2 decreasing sounds to  
start near week 15.

BD plastics MF-S sounds to  
show methane increasing after  
week 15.

I totally disagree, for example,  
because CO2 concentration  
sounds to decrease until week  
28, approximately.





265  
266

267 Fig. 3. Headspace CO<sub>2</sub> and methane concentrations, in µM. An excerpt of the CH<sub>4</sub> graph,  
268 with a smaller y-axis, is available in Fig. S4.

269

270 Visualization of the plastic film surfaces after retrieval from the sediments after 40  
271 weeks did not show any clear signs of degradation or biofilm formation, on any of the  
272 film types (PE, MF-S, MF-R; Fig. S5). DAPI staining showed a low number of  
273 microbes on the plastics' surfaces. Both regular and microscopic visualization, of all  
274 types of used plastics, showed no variation in the appearance between the end to  
275 the start of the experiment.

276

277

278





It'd be very interesting to include a text in this manuscript to approach that BD-plastic films used here were never used before, i.e. they were new and had never been used for agriculture. Thus, their degradation had not started yet. Mulch film materials that have been transported from fields to environments, as highlighted and reported in this paper, started their early degradation when they were still in the crops. Which means that if the authors had used BD-plastic samples collected from some plantation system (crop) at the end of its cycle, probably the results would reflect better the likely BD-plastic transportation from agricultural sources to freshwater of lake sediments. Then, authors must discuss what I exposed here in their manuscript.

## 279 Discussion

280

281 The biodegradability of BD-plastic films depends largely on the match between their  
282 composition and the environment. In soils, BD mulch films have been shown to  
283 biodegrade successfully (Li et al., 2014; Sintim et al., 2020). However, due to this  
284 required match between the properties of the material and the in situ conditions for  
285 biodegradation, transport of BD mulch films to aquatic environments could result in a  
286 buildup of material, as is suggested by our results. The biodegradability of the mulch  
287 films investigated in this research is in strong contrast to the biodegradability of these  
288 films under previously investigated soil conditions. Whereas within several soils,  
289 substantial degradation of the incorporated BD-plastic films was observed (11-98%  
290 after 12 months (determined by 'percentage of area remaining', (Li et al., 2014); 12-  
291 23% degradation of powdered BD-plastics after 28 days (determined by weight loss;  
292 Adhikari et al., 2016)), no biodegradation could be observed in our sediment  
293 experiments, neither based on produced CO<sub>2</sub> nor from visual analysis by  
294 microscopy. Similar conclusions were drawn based on a 100-day marine, coastal  
295 zone sediment experiment (Nauendorf et al., 2016), even though biofilm growth on  
296 biodegradable plastic bags (composed of biodegradable polyester and corn starch)  
297 was observed in that study. Freshwater BD-plastic degradation studies are more  
298 rare, and are focused on biofilm formation, rather than on biodegradation rates  
299 (Morohoshi et al., 2018). As observed by Nauendorf et al. (2016), biofilm formation  
300 and degradation are not necessarily related. Most marine sediment studies combine  
301 a biofilm assessment with visual assessments of degradation, but don't quantify the  
302 degradation, neither respiration nor fragmentation. Quantification of degradation  
303 percentages based on assessment of the remaining plastics is difficult, as  
304 microparticles, that form as a result of physical breakdown, are difficult to recover  
305 and quantify (Wei et al., 2021). Furthermore, weight assessments are often  
306 unreliable, as microbial biomass, attached to the plastics is difficult to remove  
307 completely without disturbing the plastic mass itself (Nauendorf et al., 2016).

308

309 The BD mulch films used in this study were designed to degrade in agricultural soils.  
310 A major difference between aquatic sediment conditions and soils may be the  
311 oxygen availability: due to the low diffusion speed of oxygen through water, oxygen  
312 is limiting in lake sediments, despite oxygen-rich overlying water. Lake Lucerne,  
313 where the sediments used in this study were collected, is an oligotrophic lake, with a  
314 high oxygen availability in the bottom waters (which was also remained during the  
315 experiment duration). In Lake Lucerne sediments, oxygen has been shown to be  
316 depleted from 1.0 cm downwards (Fiskal et al., 2019). The plastics were buried  
317 shallowly in the sediments, and will, at least partly, have been surrounded by  
318 sediments containing dissolved O<sub>2</sub> (DO) in our experiments. Although exact DO  
319 penetration depths can only be determined with micro-sensors, we can assume  
320 initial oxic conditions here, based on the natural oxygen penetration depth of these  
321 sediments (Fiskal et al., 2019), the oxygen concentration in the overlying water  
322 ( $\pm 400 \mu\text{M}$ , Fiskal et al., 2019), and the disturbance caused by the pushing of the  
323 plastics into the sediments, bringing oxygen-rich water in contact with the sediment  
324 surrounding the plastic film. After the initial oxic conditions, DO may have become  
325 depleted within the originally anoxic sediment depths (> 1 cm), or shallower.  
326 Although under oxic conditions, microbial growth and substrate turnover is expected  
327 to occur more rapidly, earlier research showed similar BD-plastic biodegradation  
328 rates under oxic and anoxic conditions, despite higher microbial biomass formation



329 on BD-plastics under oxic incubation conditions (Nauendorf et al., 2016). The onset  
330 of methane production in our incubations, from week 15 onwards, paired to the  
331 stabilization of the CO<sub>2</sub> concentration, suggests anoxic conditions established after  
332 15 weeks into the experiments. Although the increase in methane concentration in  
333 our MF-S and MF-R experiments could suggest enhanced breakdown of the BD-  
334 plastics, it's timely match to the decrease in CO<sub>2</sub> production, rather suggests that the  
335 respiration of sedimentary carbon, rather than the BD-plastics carbon, is being  
336 degraded, similarly to the control and PE experiment. It is, however, interesting to  
337 note that the BD-plastics experiments experienced this oxic-anoxic conversion,  
338 whereas the control and PE plastics didn't.

339  
340 Within soil experiments, several biotic and abiotic factors affect the extent of  
341 biodegradation. As the used BD-mulch films are designed to biodegrade in soils, we  
342 also consider these factors to compare the biodegradation in the aquatic realm to  
343 that of the soils. Li et al. (2014) identified soil pH, soil temperature, and fungi  
344 abundance as potential factors for differences between degradation efficiency  
345 between sites. (Sander, 2019) suggested nitrogen limitations in soils may affect and  
346 limit BD-plastic biodegradation, as the N-poor BD-plastics solely supply the  
347 colonizing microorganisms with carbon, hydrogen and oxygen-rich compounds, and  
348 not with nitrogen. Organisms will therefore have to acquire nitrogen from different  
349 sources, which, if not sufficiently available, may limit growth and activity of the BD-  
350 plastic-colonizing and degrading organisms. (Lopardo et al., 2019) showed that the  
351 addition of BD-plastics to aquaculture eluent filters can improve nitrogen removal  
352 efficiency, by providing a N-limited carbon source. Lake Lucerne sediments have  
353 been shown to contain relatively high amounts of nitrate, up to around 10 cm depth  
354 (2 μM at 2.5 cm, 33 μM at 0.5 cm depth, (Fiskal et al., 2019). Nitrogen limitation is  
355 therefore not expected to have limited microbial colonization or growth in the  
356 experiments, although more research would be needed to determine the limiting  
357 factors for microbial growth.

358  
359 The limited mobility of soil bacteria has earlier been suggested to potentially limit  
360 colonization of BD-plastics in soils, as direct contact is required between the soil and  
361 plastics to allow for colonization (Sander, 2019). Such problems may, however, be  
362 easier overcome within aquatic environment, as the water-saturation allows for a  
363 higher mobility of microbes and an easier exchange of compounds via diffusion and  
364 flow. However, the community of microorganisms may differ strongly between soil  
365 and sediment environments, mostly due to different redox and nutrient conditions  
366 between the two environments. As the genera of microbes that are capable of BD-  
367 plastic degradation are still being investigated and discovered, especially in  
368 freshwater environments, it is not possible to simply scan the microbial community  
369 for BD-plastic degraders, and make an assessment on the potential for  
370 biodegradation based on the microbial community composition. We did not  
371 determine the microbial community composition in these experiments, as the results  
372 would most likely not have been able to provide us information on the plastic  
373 degradation: the sedimentary microbial community is highly diverse, differs strongly  
374 with depth, and contains many organisms with unknown functions (Han et al., 2020).  
375 The lack of suitable BD-plastic degrading microorganisms is, however, the most  
376 likely explanation for the large difference in BD mulch film decomposition between  
377 our sediment experiment, and previously performed soil experiments. The sediments  
378 likely lack microorganisms that produce hydrolytic enzymes (esterases) that can



379 cleave PBAT and PLA, the main polyesters in the used BD-films. When specific  
380 genes known to be associated to BD plastic degradation are identified in the future, it  
381 would be interesting to screen our incubation experiment sediments for those genes,  
382 to confirm such a hypothesis.  
383

### 384 **Conclusions**

385 Two types of biodegradable plastic mulch films showed no signs of degradation over  
386 a 10-month period within lake surface sediments, suggesting very limited  
387 biodegradability in freshwater sediment environments. These findings stress the  
388 importance of limiting the transport of plastics from agricultural soils to waterways –  
389 not only for traditional plastics, but also for soil biodegradable plastics. Given that  
390 these plastics are designed to remain in the soil rather than to be removed after use,  
391 they remain present in the natural environment until complete degradation. The  
392 extend of the transport of BD mulch films from soils to waterways and lakes is up to  
393 this point unknown. Our results show it is of high importance to investigate how  
394 much of the applied biodegradable mulch films are transported to aquatic  
395 environments, to be able to assess the potential pollution of lakes and coastal areas  
396 by the current and future applications of biodegradable plastic mulch films.  
397

### 398 **Acknowledgements**

399 We want to thank Michael Sander for his valuable contributions to the manuscript,  
400 and for providing the biodegradable plastic mulch films. We thank Patrick Kathriner  
401 and Kathrin Baumann for help in the lab and field, as well as Natsumi Maeda for help  
402 with the incubation experiments.  
403

### 404 **Competing interests**

405 The contact author has declared that neither they nor their co-authors have any  
406 competing interests.  
407  
408  
409



410 **References**

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